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Escherichia coli in retail processed food

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SUMMARY

Four thousand two hundred and forty six samples of retail processed food were examined for the presence of *Escherichia coli*. Overall 12% of samples contained this organism, cakes and confectionery being more frequently contaminated (28%) than meat and meat based products (9%). Contamination was more frequent in the summer months than in the colder weather and 27% of the contaminated foods contained $> 10^3$ *E. coli*/g. *E. coli* from meat and meat based products were more commonly resistant to one or more antibiotics (14%) than were confectionery strains (1%). The significance of these findings in relation to the *E. coli* population of the human bowel is discussed.

INTRODUCTION

Food may become contaminated with *Escherichia coli* in a variety of ways. Animal carcases are regularly contaminated with the organism from the animal's bowel, even under extremely good slaughter house conditions (Shooter *et al.* 1970; Howe, Linton & Osborne, 1976; Linton *et al.* 1977*a*, *b*). Indirect contamination with the organism may also occur when contaminated water is used for the irrigation of growing crops, in food processing or during flooding. Further cross-contamination may occur during the handling of food; in this situation the *E. coli* strains could be derived initially from either human or animal faeces.

Previous work has shown that hospital, school and canteen food is regularly contaminated with *E. coli* (Shooter *et al.* 1971) the numbers present being such as to enable the ingested organisms to be detectable in the faeces. In addition, changes in the faecal flora of medical patients due to the ingestion of *E. coli* in food have been demonstrated (Cooke, Ewins & Shooter, 1969; Cooke *et al.* 1970). As *E. coli* is the commonest cause of both community-acquired and hospital-acquired infection in hospitals (Report, 1980) and as the patient's own bowel is the source of most *E. coli* infections (Gruneberg, Leigh & Brumfitt, 1968) the nature of the coliform population of the bowel is of importance.

Colonization of the human bowel by strains of animal origin may have further implications. The widespread use of antibiotics in animals has resulted in many *E. coli* strains from these sources being resistant to one or more antibiotics. It has been shown that, via the food chain, such antibiotic-resistant strains may reach

the human bowel (Linton *et al* 1977*c*) and that transfer of antibiotic resistance plasmids may occur to the normal *E. coli* of bowel flora (Smith, 1969; Bettelheim *et al.* 1977). A study of *E. coli* carrying these R plasmids (Petrocheilou & Richmond, 1976) indicated the existence of a general pool of R-plasmid carrying animal and human *E. coli* which after ingestion in contaminated food, will also be found in stool specimens and it was suggested will thus possibly become associated with subsequent infections.

Although there is some information about canteen food little is known about *E. coli* contamination of other types of food. For these reasons we have examined retail processed food to determine levels of *E. coli* contamination and have also determined the antibiotic sensitivity of the strains isolated.

MATERIALS AND METHODS

Source and nature of samples

Between October 1980 and September 1981 4246 processed foods obtained by local environmental health officers from either the despatch point of manufacturing premises or from retail outlets, were examined for *E. coli*. The foods could be placed into four groups: 2940 samples of cooked meats and meat-based products (tongue, ham, roast beef, pork pies etc.); 832 cakes and confectionery items (particularly cream and custard filled products); 300 cooked shellfish and 174 miscellaneous products.

Initial screening for E. coli

10–20 g of food was homogenized with nine volumes (w/v) of sterile quarter strength Ringer solution using a Colworth 'Stomacher' Lab. Blender 400. From this initial 10^{-1} dilution, 1 ml (=0·1 g) was added to a tube of single strength MacConkey broth purple (Oxoid CM5a). Tubes in which acid and gas were produced after 48 h incubation at 37 °C were presumptively considered to contain coliform organisms. These tubes were subcultured to modified Schuberts medium (Fennell, 1972) and incubated overnight at 44($\pm$0·5) °C. Tubes in which gas and indole were produced were considered to contain *E. coli*.

Enumeration and identification of E. coli

Food from which *E. coli* were isolated were tested further to determine the number present. The method of enumeration was an extension of the method described above. Further dilutions of 10^{-1}, 10^{-2} and 10^{-3} were transferred into tubes of single strength MacConkey broth purple (Oxoid CM5a). Incubation and confirmation of *E. coli* was as described.

The number of *E. coli* per gram of food was determined as follows: 10–100, *E. coli* present in both 1 ml aliquots of 10^{-1} dilutions but not found in both 10^{-2} dilutions; 100–1000, *E. coli* present in both 1 ml aliquots of 10^{-2} dilutions but not found in both 10^{-3} dilutions; > 1000, *E. coli* present in both 1 ml aliquots of 10^{-3} dilutions. The identity of the organisms isolated was confirmed using API 20E.

Determination of antibiotic sensitivity

Four hundred and forty eight of the isolates were examined for antibiotic sensitivity and an additional 82 isolates from raw food were also examined.

Table 1. *Frequency of isolation of* E. coli *from processed foods*

Type of food	No. of samples tested	No. in which *E. coli* found (percentage)
Meat & meat-based products	2940	261 (8·9)
Cakes and confectionery	832	232 (28·0)
Seafoods	300	33 (11·0)
Miscellaneous	174	9 (5·2)
Total	4246	535 (12·6)

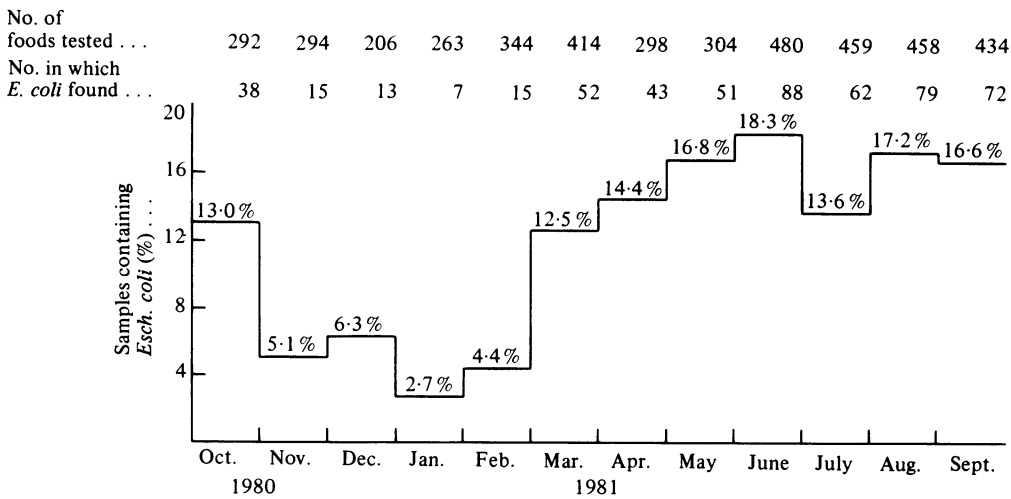

Fig. 1. Monthly incidence of *E. coli* in retail, processed food samples.

Sensitivity testing was done on STA agar (Oxoid CM471) using the disc diffusion method of Stokes (1975) and the rotary plating inoculation technique of Pearson & Whitehead (1974).

The disc strengths were ampicillin 10 μg; chloramphenicol 10 μg, gentamicin 10 μg, kanamycin 30 μg, nalidixic acid 30 μg, nitrofurantoin 200 μg, streptomycin 10 μg, sulphafurazole 100 μg, and tetracycline 10 μg. After overnight incubation at 37 °C the zones were measured and classed as follows: sensitive, zone size (from edge of disc) equal to or greater than the control, or if less than the control, then not by more than 3 mm; intermediate, zone size (from edge of disc) greater than 3 mm, but smaller than the control by more than 3 mm; resistant, zone size 3 mm or less from edge of disc.

RESULTS

Frequency of isolation

These results are shown in Table 1. *E. coli* was more commonly isolated from cakes and confectionery than from meat products. During the summer months frequency of contamination with *E. coli* was higher than in the cold weather (Fig. 1).

Table 2. *Numbers of* E. coli *found in 535 processed foods*

Type of food	Number containing *E. coli*	Number of samples with count/g (percentage) 10–100	100–1000	> 1000
Meat & meat-based products	261	134 (51·2)	52 (19·9)	75 (28·7)
Cakes and confectionery	232	114 (49·2)	54 (23·3)	64 (27·5)
Seafoods	33	27 (82·0)	4 (12·1)	2 (6·1)
Miscellaneous	9	5 (55·5)	2 (22·25)	2 (22·25)
Totals	535	280 (52·2)	112 (21·0)	143 (26·7)

Table 3. *Antibiotic resistance of* E. coli *strains isolated from raw and processed foods*

Type of food	Number of strains tested	Number resistant to one or more antibiotics (percentage)
Raw Foods		
Meat	68	8 (11·8)
Poultry	5	1
Seafoods	9	4
Totals	82	13 (16·0 %)
Processed foods		
Meat & meat-based products	222	31 (14·0)
Cakes and confectionery	190	2 (1·05)
Seafoods	28	2 (7·2)
Miscellaneous	8	0 (0·0)
Totals	448	35 (7·8)

The numbers of *E. coli* found in each type of food are given in Table 2; 27 % of the samples had *E. coli* counts greater than 10^3 per gram.

Antibiotic sensitivity

In Table 3 the sensitivity to antibiotics of *E. coli* strains isolated from raw and processed food is recorded. Of the 82 strains from raw food, 13 (16 %) were resistant to one or more antibiotics, while 35 strains (8 %) isolated from processed foods were resistant. There was a marked difference in the occurrence of resistant strains in the two main categories of processed foods examined. Of the 222 strains isolated from meat and meat products, 31 (14 %) showed resistance to antibiotics, whilst only 2 (1 %) of 190 strains isolated from cakes and confectionery products were found to be antibiotic resistant.

The number and type of antibiotics to which individual strains were resistant are shown in Table 4. The resistance patterns of the strains are given in Table 5.

Table 4. *Sensitivity of* E. coli *strains to ampicillin, chloramphenicol, gentamicin, kanamycin, nalidixic acid, nitrofurantoin, streptomycin, sulphafurazole and tetracycline*

Source	Number of strains tested	Strains resistant to following numbers of antibiotics										Number of resistant strains	Number of strains resistant to								
		0	1	2	3	4	5	6	7	8	9		A	C	G	K	N	Nit	S	Sul	T
Raw foods																					
Meat	68	60	1	3	2	2	0	0	0	0	0	8	2	0	0	0	0	0	7	7	5
Poultry	5	4	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0
Seafoods	9	5	2	0	1	0	0	1	0	0	0	4	2	1	0	1	0	0	2	4	1
Totals	82	69	4	3	3	2	0	1	0	0	0	13	4	1	0	1	0	0	9	12	6
Processed foods																					
Meat & meat-based products	222	191	11	12	4	3	1	0	0	0	0	31	6	1	0	0	0	2	17	19	19
Cakes and confectionery	190	188	1	0	1	0	0	0	0	0	0	2	0	1	0	0	0	0	1	1	1
Seafoods	28	26	0	1	1	0	0	0	0	0	0	2	0	0	0	0	0	0	2	1	2
Miscellaneous	8	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Totals	448	413	12	13	6	3	1	0	0	0	0	35	6	2	0	0	0	2	20	21	22

A, ampicillin; C, chloramphenicol; G, gentamicin; K, kanamycin; N, nalidixic acid; Nit, nitrofurantoin; S, streptomycin; Sul, sulphafurazole; T, tetracycline.

Table 5. *Resistance patterns of* E. coli *strains isolated from raw and processed foods*

Antibiotic combination	Raw foods				Processed foods			
	Meat	Poultry	Sea foods	Total	Meat and meat products	Cakes and confectionery	Cooked seafoods	Total
A	—	—	—	—	3	—	—	3
C	—	—	—	—	—	1	—	1
S	—	—	—	—	1	—	—	1
Sul	—	1	2	3	—	—	—	—
T	1	—	—	1	7	—	—	7
Nit.Sul	—	—	—	—	1	—	—	1
S.Sul	3	—	—	3	7	—	—	7
S.T	—	—	—	—	1	—	1	2
Sul.T	—	—	—	—	3	—	—	3
S.Sul.T	2	—	—	2	4	1	1	6
A.S.Sul	—	—	1	1	—	—	—	—
A.S.Sul.T	2	—	—	2	2	—	—	2
Nit.S.Sul.T	—	—		—	1	—	—	1
A.C.S.Sul.T	—	—	—	—	1	—	—	1
A.C.K.S.Sul.T	—	—	1	1	—	—	—	—
Total	8	1	4	13	31	2	2	35

A, ampicillin; C, chloramphenicol; G, gentamicin; K, kanamycin; N, nalidixic acid; Nit, nitrofurantoin; S, streptomycin; Sul, sulphurazole; T, tetracycline.

DISCUSSION

The results obtained in this survey show that the consumption of retailed processed food will regularly result in the ingestion of *E. coli*. The frequency of contamination found is higher than we expected, and raises the question as to its significance. Ingested *E. coli* may become of importance if they have a particular ability to affect the bowel flora, or cause disease in the bowel itself or elsewhere in the body. In the investigation reported here we have considered only the numbers and antibiotic resistance of the ingested strains.

Of the 535 contaminated specimens of food 143 (27 %) contained $> 10^3$ *E. coli*/g. For many of the foods examined, consumption of a 100 g portion could be expected so that 10^5 organisms or more would be ingested. This is sufficient to result in bowel colonization (Cooke, Hettiaratchy & Buck, 1972).

One unsatisfactory feature of this colonization of the human bowel is that man may acquire antibiotic-resistant strains. The use of antibiotics in animal husbandry has resulted in salmonellae which possess multiple antibiotic resistance reaching the human population (Anderson, 1965). It is known that *E. coli* of animal origin can become antibiotic resistant in the same way. In this survey some of the *E. coli* from raw food, which was predominantly raw meat, and from processed meat and meat-based products, showed multiple antibiotic resistance, occasional strains being resistant to 4, 5, or 6 antibiotics. By marked contrast the strains present in cakes and confectionery were almost entirely antibiotic sensitive. (Table 4). This difference suggests that the sources of the strains are also different. From the results it seems possible that the strains on the processed meats originate from

antibiotic resistant strains of animal origin present on the raw meat, unlike the confectionery strains for which there are likely to be other sources. Further investigations in the production areas are necessary to elucidate these sources.

Comparison of the resistance patterns of the strains from raw and processed meats further suggests that the antibiotic resistant strains on processed meats are present as a result of cross-contamination from raw meat. Strains from raw meat and poultry frequently showed resistance to streptomycin, sulphonamides and tetracycline either alone or in various combinations. Resistance to tetracycline (61 %), sulphonamides (61 %), and streptomycin (55 %) was also commonly found in the strains from processed meat and meat products (Table 4 and 5). These findings agree with other published studies carried out on *E. coli* of both animal and human origin. In a survey carried out on *E. coli* isolated from the faeces of pigs and chickens, Smith & Lovell (1981) showed that 64 % of chicken isolates were resistant to sulphonamides, 32 % to tetracycline, and 10 % to streptomycin. In pigs the pattern was similar; 40 % were resistant to tetracycline, 34 % to sulphonamides, and 27 % to streptomycin. In Great Britain, Linton (1977) found *E. coli* strains in meat from calves, pigs and poultry, 50–70 % of which carried resistance determinants to tetracyclines. Datta (1969) studying the bowel bacteria of patients before and after admission to a London hospital, found that resistance to tetracycline and sulphonamides was commonly present, either alone or in combination, and in either case was often accompanied by resistance to streptomycin.

There is much controversy over the extent to which these non-pathogenic *E. coli* of animal origin act as a source of R-factor-bearing strains for man. It has been suggested that the most probable route by which this could occur is by the ingestion of meat foods contaminated with R-factor-bearing *E. coli* of animal origin (Cook *et al.* 1970; Shooter *et al.* 1970; Cooke *et al.* 1971; Howe, Linton & Osborne, 1976). There is evidence that some of these strains can colonize the human gut and transfer the resistance pattern to the normal intestinal flora, particularly in the presence of antibiotics, although it has been shown that colonization does not easily occur (Smith, 1969; Cooke *et al.* 1972; Bettelheim *et al.* 1977; Linton *et al.* 1977*c*). Others may transfer their R factors to resident Gram-negative flora during passage through the gut or pass their R-plasmids on to more virulent members of the enterobacteriaceae (Hartley & Richmond, 1975).

This work indicates another source, retailed processed foods, by which *E. coli* may be ingested by the general population. The numbers present are sufficient to result in colonization and some of the strains present have multiple antibiotic resistance. More work is in progress to determine whether the strains can transfer their antibiotic resistance to other organisms. Other properties are also being studied so that their ability to cause disease in the bowel and elsewhere may be defined.

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